The Microscope

and some hints on

How To Use It.

E. Leitz

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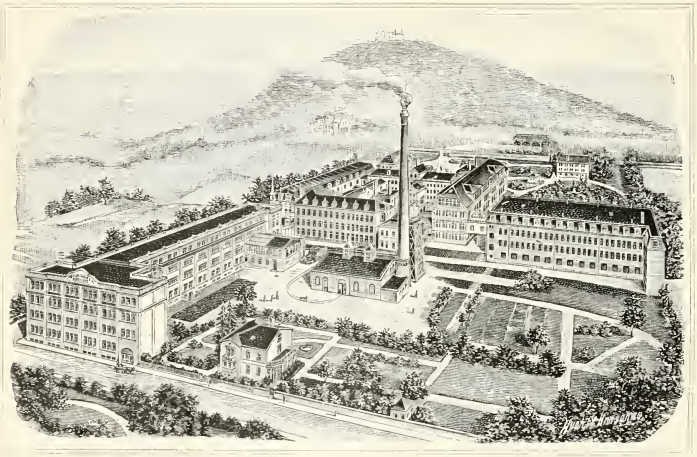

Leitz Optical and Mechanical Works.

The Optical Works of E. Leitz were established in 1849 by C. Kellner. Originally its operations were confined to the manufacture of microscopes, but gradually other objects were added to the scope of its activities. Apart from microscopes for general and specialized study the establishment is now occupied with the manufacture of photomicrographic apparatus, microtomes, appliances for optical projection and drawing, photographic lenses, field-glasses and so forth.

Of the aggregate number of microscopes produced throughout the world, the Leitz Optical Works supply the principal share. Since 1875 about 120,000 microscopes and 50,000 oil-immersion lenses have been turned out in Wetzlar, the annual production of microscopes being now about 10,000.

Besides the works at Wetzlar the firm has branches at **Berlin N.W.,** 45 Luisenstrasse; **Frankfort o./M.,** 24 Neue Mainzerstrasse; **St. Petersburg,** 11 Woskressenski; **London, W.,** 9—15 Oxford Street; **New York,** 30 East 18th Street; **Chicago,** 1923 Ogden Avenue.

Introduction.

In this little booklet entitled "The Microscope and some hints on how to use it" we do not in any sense pretend to deal exhaustively, or even in detail, with the working principles of the microscope. Its humble purpose is to furnish a first introduction to a more advanced use of the microscope and to offer a guide to those whose work involves an occasional use of the instrument. To the research worker who devotes himself primarily to microscopic observations the booklet presents only so much practical knowledge as will ensure a satisfactory measure of success in its use.

After a brief review of the optical and mechanical components of the microscope and a short explanation of the process by which the microscope furnishes a magnified image of the object, we shall proceed to elucidate in detail such matters as the aperture of an objective, its resolving power and magnification. Next we shall say a little on the construction of condensers, objectives and eyepieces, and conclude with a few remarks on the measurements which can be made with the microscope.

We shall endeavour to be as brief as our desire to be clear and intelligible admits of.

Electros of illustrations contained in the booklet are at the disposal of authors.

E. Leitz.

1910.

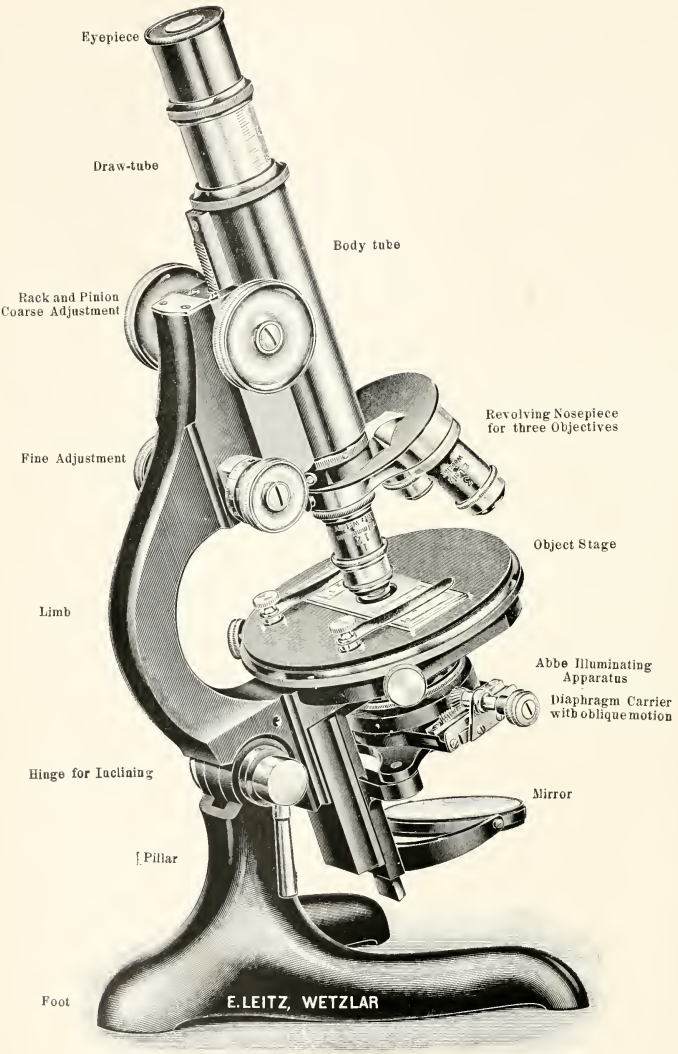
Eyepiece
Draw-tube
Body tube
Rack and Pinion
Coarse Adjustment
Revolving Nosepiece
for three Objectives
Fine Adjustment
Object Stage
Limb
Abbe Illuminating
Apparatus
Diaphragm Carrier
with oblique motion
Hinge for Inclining
Mirror
Pillar
Foot
E. LEITZ, WETZLAR

Fig. 1.

Short Description of the Microscope.

As will be seen from Fig. 1, a microscope consists of the following parts:

A. Mechanical.

1. **The Body with its Draw-tube.** The latter has a millimetre scale which shows the total length of the working tube reckoned from the shoulder of the objective screw to the eyelens of the eyepiece. The latter fits the top of the tube, whilst the lower end is provided with a screw thread for the attachment of an objective, revolving nosepiece or other screw adapter.
2. **The Limb,** especially in the more recent models, forms a convenient handle. It carries the coarse and fine focussing adjustments, of which the former is actuated by a rack and pinion, whilst the latter is of the nature of a micrometer screw.
3. **The Object Stage,** which in many stands is so arranged that it may be rotated and centred. It has an opening at the centre which admits light from the illuminating apparatus to the object.
4. **The Pillar** with a hinge for inclining the instrument.
5. **The Foot,** which is either of the horseshoe or of the English tripod pattern.

B. Optical.

1. **The Illuminating Apparatus,** consisting of a plane and concave mirror, a stop usually in the form of an iris-diaphragm, which in the larger stands is attached to a separate swing-out diaphragm carrier fitted with a rack and pinion for placing the diaphragm in an eccentric position, and finally a condenser.
2. **The Objectives.** These are either of the "dry" series, or they are immersion lenses.
3. **The Eyepieces.** There are two kinds, viz. Huyghenian and Compensating Eyepieces.

Path of Rays.

Before proceeding to describe the various parts of the microscope and to explain their respective functions it will be well to trace out the course of the rays as they pass from the mirror through the optical system, which will convey a general notion of the optical principle of the microscope.

Path of a Pencil of Light through a Microscope.

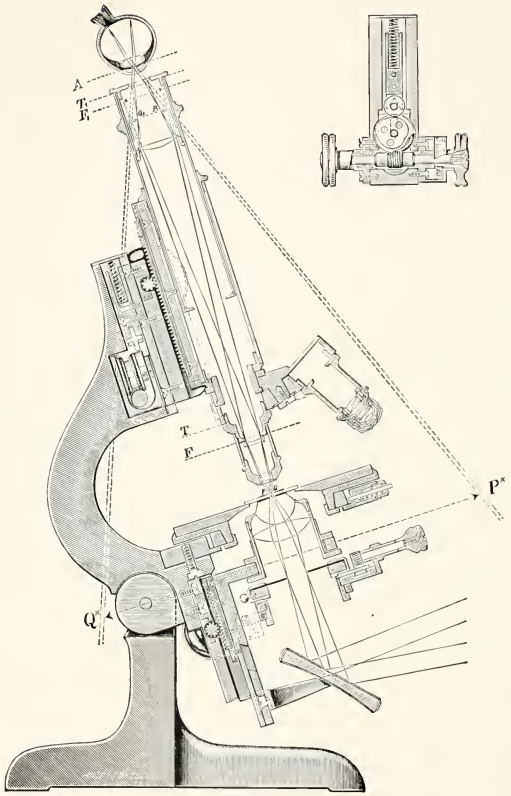

Fig. 2.

A pencil of rays, after reflection at the mirror of the illuminating apparatus, traverses in succession the following parts:

1. Condenser,
2. Object slide,
3. Cover-glass,
4. Objective,
5. Eyepiece,

also media intervening between the parts enumerated above.

The only medium which intervenes between the objective and eyepiece and again between the mirror and the condenser is air. With the so-called *dry* lenses air is likewise the medium which separates the objective and the cover-glass, with the water-immersion lenses it is water, and with the *homogeneous* oil-immersion lenses it is cedarwood-oil.

The same media occur in the space intervening between the condenser and the object-slide. Between the object-slide and the cover-glass a great variety of media may occur apart from the microscopic object. These media may be air in dry-mounted preparations, and in other prepared and mounted objects they may be any of the numerous substances used for their preservation and treatment for microscopic observation, such as water, cedarwood-oil, paraffin, Canada balsam, turpentine, alcohol, glycerine, etc.

Fig. 2* shows the path of the rays as they proceed from the mirror through a two-lens condenser, an Objective No. 3, and an Eyepiece No. II. It is represented by the two extreme rays of the pencils proceeding from the points P and Q. These points may be supposed to be situated at the extremities of the diameter of the circle which forms the boundary of the object plane, and it is further assumed that they are symmetrically placed with respect to the axis of the microscope. These extreme rays are traced backwards through the condenser to the mirror.

It will be seen that proceeding from the two points P and Q of the object two divergent pencils enter the objective. On emerging from the objective they are made to converge to points situated within the plane F_1. Passing through the lower lens of the eyepiece, the so-called field-lens, the rays of the respective pencils meet in points in the plane of the diaphragm, where they form a real image Q_1 P_1 of the object PQ. The plane of the stop, which is identical with that of the image there formed, is the focal plane of the upper lens of the eyepiece, which is known as the eye-lens, and hence the emerging rays are made up of parallel rays and reach the eye as such. An eye accommodated or corrected for infinity, as represented in the diagram, will under these conditions perceive a clearly defined image of the object, since the lens of such an eye will be able to bring the incident pencils of parallel rays to a focus on the retina, forming thereon a real image of the object.

The pencils of parallel rays meet in the plane A and there form a bright circle of light, which may be seen by holding the eye

* A coloured wall diagram, of which Fig. 2 is a reduced facsimile and which measures 44×28 inches, is supplied free of charge for teaching and lecturing purposes.

a little distance from the eyepiece. The bright circle is known as the *pupil of the eyepiece* or the *Ramsden disc.*

The plane at F is the posterior focal plane of the objective, and the plane at F_1 is the anterior focal plane of the eyepiece. The distance of these two planes is described as the *Optical Tube-length* $= \Delta$

Let f_1 be the focal length of the objective, f_2 that of the eyepiece, then the resulting focal length of the entire microscope is

$$f = \frac{f_1 \cdot f_2}{\Delta} .$$

The distance between the plane T_1, which is the upper edge of the tube and T, the shoulder of the objective collar, is known as the *Mechanical Tube-length.*

Let the pencil of parallel rays which reaches the eye be produced backwards, and at a distance of 250 mm from the line A let a line $Q^* P^*$ be drawn at right angles to the axis of the microscope, then the ratio

$$\frac{Q^* P^*}{P Q} = M = \frac{250}{f}$$

is a measure of the magnifying power of the microscope, in that this ratio shows how many times the image of the object as seen in the microscope appears enlarged as compared with the object itself, supposing the latter to be viewed at a distance of 250 mm from a normal eye.

In order that all rays which proceed from the object to the objective may reach the retina of the eye the pupil of the latter should coincide with the exit pupil of the eyepiece, as shown in the diagram.

To understand how the expression for the magnifying power $M = \frac{Q^* P^*}{P Q}$ has been arrived at we will consider the case of an object viewed by a simple lens of a focal length F. It will be easy to show by reference to Fig. 3 that its magnifying power will be $\frac{250}{F}$.

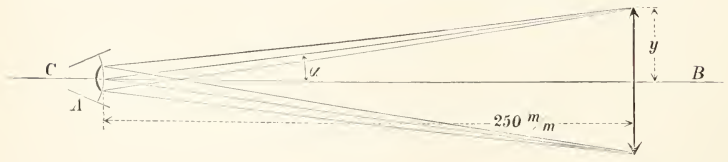

Fig. 3.

Viewing the object first with the unaided eye at a distance of 250 mm we find that half the length of the object subtends at the eye an angle α, such that

$$\tan \alpha = \frac{y}{250}.$$

If now we introduce a lens, placing the object in its focal plane, it follows that each pencil of light proceeding from points of the object, as it emerges from the lens, consists of parallel rays,

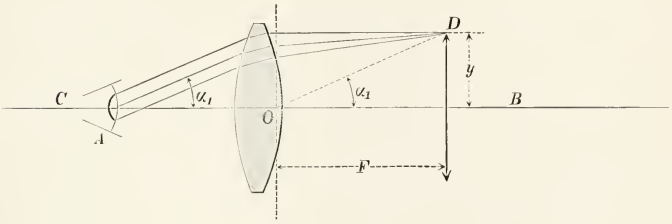

Fig. 4.

and it will be seen that the extreme emerging pencil of parallel rays which proceeds from the edge of the object includes with the axis CB of the lens the same angle as the line OD. Hence it follows from the figure that

$$\tan \alpha_1 = \frac{y}{F},$$

and the expression for the magnifying power becomes

$$M = \frac{\tan \alpha_1}{\tan \alpha} = \frac{250}{F}.$$

The Aperture.

As will be seen from Fig. 2, the object is illuminated by light focussed to a point, whilst the pencil proceeding from the illuminated object goes to form the microscopic image as perceived by the eye.

The reader should carefully distinguish between the pencils which illuminate the object and those which give rise to the magnified image.

Let u be the angle included between the extreme ray of either cone of light, or field, and the axis of the microscope, and let n be the refractive index of the medium in which the object is mounted, then the quantity

$$a = n \cdot \sin u$$

is known as the *numerical aperture* of an optical system. If, for instance, the mounting medium be cedarwood-oil having a refractive index of $n = 1.515$, a numerical aperture of $a = 1.40$ will render it possible to admit rays whose inclination to the axis may be found from the relation

$$\sin u = \frac{1.40}{1.515} = 0.924,$$

whence it follows that in this case

$$u = 67^0 6.$$

The subjoined Table gives for various media the numerical apertures corresponding to a number of angular apertures.

Numerical Apertures.

	$n = 1.00$ Air	$n = 1.33$ Water	$n = 1.52$ Immersion oil	$n = 1.66$ Monobrom-naphthalene
$2u = 10^0$	0.09	0.12	0.14	0.15
20^0	0.18	0.24	0.26	0.29
30^0	0.26	0.35	0.40	0.43
40^0	0.34	0.46	0.52	0.57
50^0	0.42	0.56	0.64	0.70
60^0	0.50	0.66	0.76	0.83
70^0	0.57	0.76	0.87	0.95
80^0	0.64	0.85	0.98	1.07
90^0	0.71	0.94	1.07	1.17
100^0	0.77	1.02	1.16	1.27
110^0	0.82	1.09	1.24	1.36
120^0	0.87	1.15	1.32	1.44
130^0	0.91	1.20	1.38	1.50
140^0	0.94	1.25	1.43	1.56

In applying these data a distinction should be drawn between the illuminating and the image-forming cone of rays, that is to say between the aperture of the condenser and that of the objective. The subjoined illustration (Fig. 5) may assist us to a just appreciation of the significance of the aperture of the microscope objective.

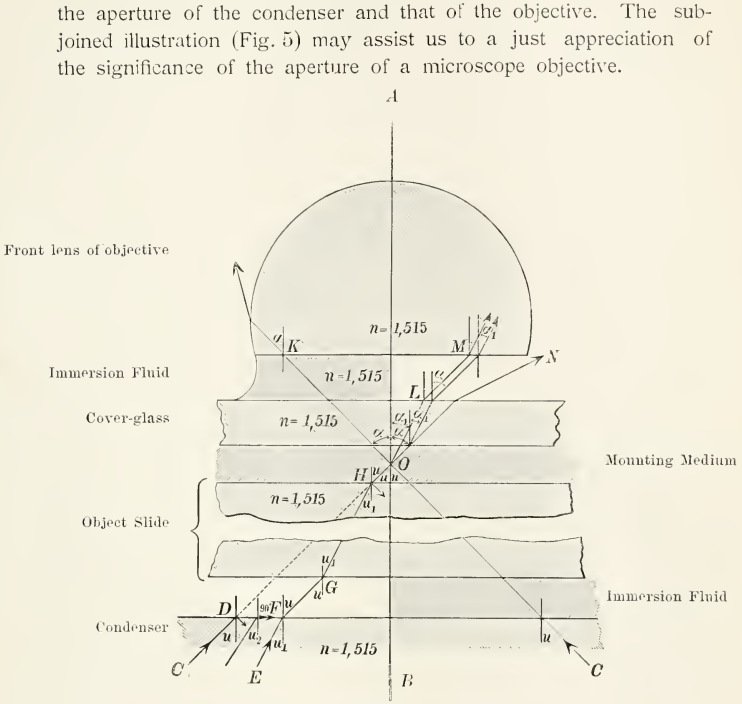

Fig. 5.

1. The Aperture of the Condenser. Let $A\ B$ be the optical axis of the microscope and let O mark the position of the object. It will be seen that the ray $C\ O$ passes from the condenser without refraction to the object, and hence retains the same inclination u to the axis which it had within the condenser. The aperture of the condenser is accordingly

$$a = n \,.\, sin\ u, \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

n being the refractive index of the mounting fluid, whilst that of the object slide and the fluid intervening between the object slide and the front lens of the condenser as well as that of the lens itself should not differ appreciably from the refractive index n of the mounting medium. When these conditions obtain, as represented in Fig. 5, the method of observation is said to involve the principle of *homogeneous immersion*.

When the two fluids are absent and air takes their place, as indicated in the left half of the diagram, then it becomes impossible for any ray $C_1 D$ occupying a symmetrical position to the ray $C O$ with respect to the axis of the microscope to emerge from the condenser by refraction, being prevented from so doing by total reflection. Let $E F G H O$ be that ray which, after traversing the object slide and when it reaches O, includes with the axis the same angle as the original ray $C O$. In this case the numerical aperture of the condenser becomes

$$a_1 = n \cdot \sin u_1, \quad \ldots\ldots\ldots (2)$$

u_1 being the angle which the emerging ray forms with the axis at F. Since the refractive index of the object slide is the same as that of the condenser lens and also since all refracting surfaces are parallel it follows from the law of refraction that

$$\frac{\sin u}{\sin u^1} = n$$

$$\text{hence } a_1 = n \sin u_1 = \sin u \quad \ldots\ldots\ldots (3)$$

Combining this result with equation (1) we obtain the relation

$$\frac{a_1}{a} = \frac{\sin u}{n \cdot \sin u} = \frac{1}{n} \quad \ldots\ldots\ldots (4)$$

If, accordingly, in the place of a fluid air intervenes between the object and the object slide, and likewise between the object slide and the condenser the aperture of the condenser becomes reduced to $\frac{1}{n}$ th its original value.

The intensity of the illumination which the object O receives through the condenser is proportional to the square of the aperture. Hence when air takes the place of a fluid medium the brightness of the image diminishes in the ratio of

$$\frac{a_1^2}{a^2} = \frac{1}{n^2} \quad \ldots\ldots\ldots (5)$$

Further it will be seen from Fig. 5 that the angle contained between the ray $F G$ and the axis is the same as that contained between $H O$ and the axis, both being equal to u. Since the greatest value of sin u cannot exceed 1 it follows by (3) that the numerical aperture of the condenser cannot exceed 1. In the case of a condenser whose front lens is separated from the object slide by air the maximum is reached when $u = 90^0$. Substituting in this limiting case u_2 for u_1 in equation (4) we may write the relation thus:-

$$\sin u_2 = \frac{\sin 90^0}{n} = \frac{1}{n} \quad \ldots\ldots\ldots (6)$$

This limiting value u_2 is known as the *critical angle of total reflection* for glass as used in the condenser lens with respect to air. Any rays whose angle of incidence at the plane of contact of glass and

air is greater than u_2 do not take part in the illumination of the object, since they are all reflected back into the condenser, as shown at D.

From this it will be seen that a condenser cannot furnish an aperture greater than 1 unless there be an appropriate medium other than air between the condenser and the object slide and also between the cover-glass and the object slide, in which case the condenser performs as an *immersion condenser*.

The presence of air in any of the spaces intervening between the condenser and the front lens of the objective renders it impossible for the condenser, of whatever construction, to have a numerical aperture exceeding unity. The presence of air between the condenser and object slide causes the ray at D to undergo total reflection, and if the mounting medium be air reflexion occurs at H.

2. Aperture of the objective. The aperture of the objective depends upon the obliquity of the extreme rays which, after proceeding from the object may yet enter the objective and also upon the refractive indices of the various intervening media, such as the mounting medium, cover-glass, immersion fluid, etc., which the rays have to traverse.

Where, as in the diagram, the mounting medium at O is oil, any ray OK proceeding from the object at an angle α with respect to the axis enters the cover-glass without undergoing refraction, and if the immersion fluid be likewise oil it enters the objective also without being refracted since the refractive indices of the oil and the cover-glass do not differ appreciably, being equal to $n = 1.515$. In this case the aperture of the objective may be expressed by the relation

$$a = n \sin \alpha \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

If a dry objective be brought to bear upon an object mounted in air, as represented in the right half of the diagram, in that case a ray proceeding from the object at the same angle α will pass through the cover-glass and emerge from it with unchanged obliquity. The resulting aperture will now be $a_1 = \sin \alpha_1$, and since all intervening surfaces are parallel it follows from the law of refraction that

$$a_1 = \sin \alpha = n \sin \alpha_1 \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

n being again the refractive index of the cover-glass, which is identical with that of the immersion oil.

Combining the relations (1) and (2) we obtain the relation

$$\frac{a_1}{a} = \frac{\sin \alpha}{n \sin \alpha} = \frac{1}{n} \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

It will be seen that as in the case of the condenser the aperture becomes n times smaller when air takes the place of immersion oil.

If now oil be employed as a mounting fluid then a ray proceeding from the object at an angle α enters the cover-glass without being refracted. At the interface of the cover-glass and air it is, however, refracted in such a way as to cause it to proceed to N, and hence it cannot enter the objective. It will thus be seen that the angle α at which a ray leaves the object represents the obliquity of the extreme ray which may enter the objective. In the diagram such a ray is represented by $O\,L\,M$. In this case the aperture is $n \sin \alpha_1$ and by (2) this is equal to $\sin \alpha$, that is to say the aperture of a *dry* objective is not affected by the presence of a mounting medium.

Since the obliquity of a ray as it emerges from the cover-glass may not exceed 90° it follows from (2) that the greatest possible value of the numerical aperture of a *dry* lens is unity. In practice 0.95 represents the highest aperture which has been realized.

Denoting the refractive index of the mounting medium by n_1, that of the cover-glass by n_2, that of the immersion fluid by n_3, and the corresponding angles of obliquity by α_1, α_2, α_3 (Fig. 6), it follows from the laws of refraction, since all the refracting surfaces are parallel, that

$$n_1 \sin \alpha_1 = n_2 \sin \alpha_2 = n_3 \sin \alpha_3 \quad . \quad . \quad . \quad . \quad (4)$$

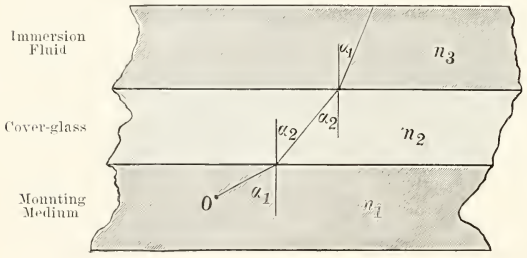

Fig. 6.

This constant product is nothing more or less than the aperture a.

If $n_3 = 1$, i. e. if air take the place of an immersion fluid, then of all the rays proceeding from the object those only may enter the objective with respect to which α_1 has a value such that $n_1 \sin \alpha_1 \leq 1$. All rays of greater obliquity undergo total reflection at the surface of the cover-glass. Since by (4) a cannot be greater than 1 unless n_1, n_2, and n_3 are also greater than 1 it follows immediately that an objective having an aperture exceeding 1 cannot be used to its full working capacity unless the refractive indices of the mounting media and the immersion fluid exceed 1.

The practical significance of the numerical aperture of an objective lies in the fact that the quantity of light which passes from the objective and so reaches the eye is proportional to the square of the aperture and in that it determines the resolving power of an objective, as we shall endeavour to show on page 18.

We will conclude this section with a note on the evaluation of the numerical aperture of an objective or condenser.

The aperture was defined as the product of the refractive index into the sine of the angle contained between the axis and the ray under consideration. Since, however, the condenser and the objective are separated by six refracting surfaces and seven different media, as shown in Fig. 5, all of which may have different refractive properties, the question arises as to whether this does not introduce an element of confusion.

From equation (4) it will at once be seen that we may select any one medium and compute the numerical aperture by considering the extreme ray within that medium which passes through all the media from the object to the objective, or from the condenser to the object, as the case may be. We may accordingly select any one of the given media and multiply its refractive index into the sine of the angle contained between the axis of the microscope and the extreme ray which traverses the medium, and in every case the numerical value of the aperture will necessarily be the same.

Resolving Power.

Having explained the general meaning of the aperture of an objective, or a condenser, we will proceed to give a brief exposition of what is understood by resolving power.

The resolving power of an objective is a matter of special interest in connection with the study of objects having very fine structural details, such as diatoms.

The simplest example of an object of this kind is furnished by a grating consisting of alternate transparent and opaque bars, e. g. a silvered plate of glass having a series of lines ruled upon it at equal intervals.

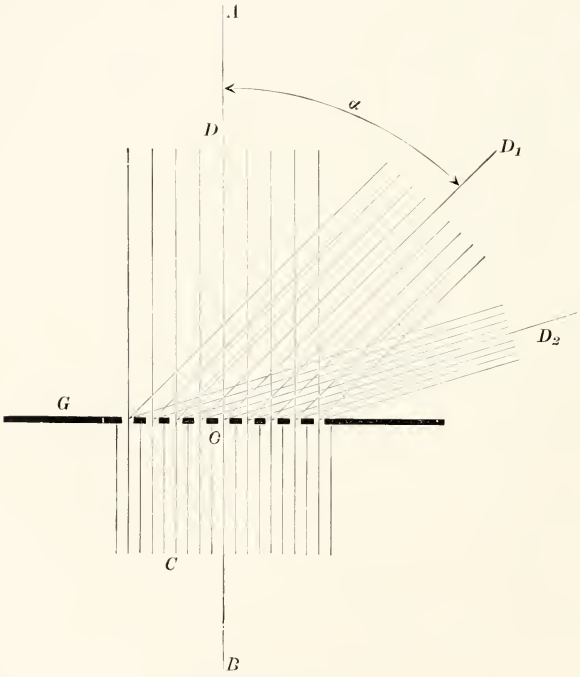

Fig. 7.

In Fig 7 let G be a sectional view of a grating placed with its bars at right angles to the plane of the paper and let $A\,B$ be the axis of the microscope. Also let the iris-diaphragm below the

condenser be covered with a ground glass disc and let the iris-diaphragm itself be closed down to an extremely small opening. Under these conditions the illuminated ground-glass disc will furnish an approximation to a point source of light.

Since the ground glass disc is approximately situated in the posterior focal plane of the condenser it follows that the light which proceeds from the very small area situated in the axis of the microscope will emerge from the condenser in the form of a pencil of rays which are parallel to the axis of the microscope. This pencil is represented in Fig. 7 by CD. In investigating this pencil we will first suppose that the light supplied to the microscope is purely monochromatic.

The pencil CD passes straight through the grating. In accordance with a well known principle in physical optics the grating transmits a series of other pencils OD_1, OD_2, etc. by diffraction. These pencils, of which two only (first and second order) are shown in the figure, are symmetrically disposed on either side of the axis OD. The diffracted pencils have a definite direction and intensity, which are determined by the nature of the grating, the wave-length of the incident light, and the nature of the medium which contains the grating, i. e. the mounting medium.

It is easy to see that even the diffracted pencil of the first order may be inclined to the axis to such an extent that it cannot enter a given objective, in which case the latter receives the direct pencil of rays OD transmitted through the grating. Under these circumstances the microscope fails to disclose the structure of the grating and hence the eye will see through the microscope merely a uniformly illuminated field.

We shall now have no difficulty in appreciating the fact that we cannot expect to see the outlines of the grating unless in addition to the direct light we cause a pencil of diffracted light of at least the first order to enter the objective.

Now, the theory of diffraction furnishes a relation from which we may determine the angle α_1 at which the diffracted pencil of the first order emerges from the grating. Let λ be the wave-length of the incident light measured in air, also let n_1 be the refractive index of the mounting medium and e the constant of the grating, i. e. the distance between the centres of two consecutive bars or gaps of the grating.

By the formula referred to, viz.

$$n_1 \sin \alpha_1 = \frac{\lambda}{e}, \quad \ldots \ldots \ldots \quad (1)$$

it will be seen that the expression on the left is identical with that

which we had obtained for the numerical aperture a. We may accordingly write

$$a = \frac{\lambda}{e} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

From this it follows that to be able to recognise, or resolve, with light of wave-length λ the structural outlines of a grating having a constant of diffraction e we require an objective having an aperture of $\frac{\lambda}{e}$ at least.

An aperture exceeding the limit which results from the application of formula (2) enables an objective to take in diffracted pencils of the second, third, and even higher orders. In the actual microscopic image this shows itself by the increased distinctness with which the grating appears to the eye, in that the definition, that is the transition from the dark bars to the bright gaps becomes more and more clearly marked in proportion as the number of diffracted rays which are enabled to enter the objective is augmented.

Assuming that the grating is contained in air, and putting $n = 1$, then equation (1) takes the form

$$\sin \alpha_2 = \frac{\lambda}{e} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

Equating the expression on the left of the equations (3) and (1), as obviously we may do, we find

$$n_1 \sin \alpha_1 = \sin \alpha_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

$$\text{and hence } \alpha_1 < \alpha_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

From the figures it will be seen that the diffracted pencils are arranged symmetrically on either side of the axis $A\,B$ in the form of a fan. From equation (5) we learn that when the grating is enclosed in a denser medium the fan-like system of diffracted pencils will close up more and more as the refractive index of the medium increases.

This provides the means of causing a larger number of pencils to enter the objective, so as to increase its resolving power. This fact points at once to the significant influence which the mounting medium exercises upon the resolution of finely marked objects.

Supposing that the constant of the grating is so small that the evaluation of the expression furnishes a greater numerical aperture a than that realised in any available objective, then it follows from what has been shown above that none but the direct pencil $O\,D$ can enter the objective, and hence the microscope will fail to disclose the structural details of the grating.

To achieve the desired result in this case we may select an extra-axial pencil on the illuminated disc. In practice this is done by displacing the small opening of the iris-diaphragm laterally with

the aid of the rack and pinion movement of the diaphragm carrier, and we will suppose this displacement to be at right angles to the grating.

This displacement, since the illuminated ground glass disc is approximately at the focus of the condenser has the effect of sending through the grating a pencil O D of parallel

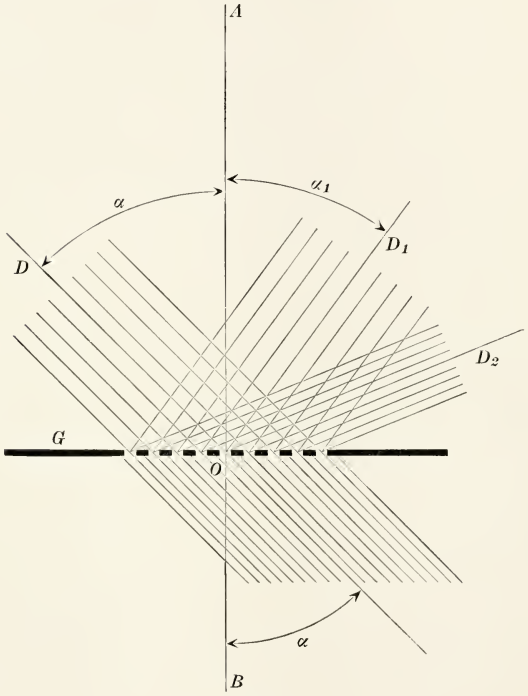

Fig. 8.

rays inclined at an angle α to the axis of the microscope (Fig. 8), and it will give rise to the formation of diffracted pencils the first of which is inclined to the axis at an angle which we will denote by α_1, Then, if e_1 is the very small constant of the grating,

$$n_1 \sin \alpha + n_1 \sin \alpha_1 = \frac{\lambda}{e_1} \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

where n_1 refers, as before, to the mounting medium.

A comparison of Fig. 7, which illustrates the case of direct illumination, with Fig. 8. which represents the conditions as they obtain in oblique illumination, shows that the latter mode of illum-

ination affords the better means of sending the first diffracted pencil into the objective in addition to the direct pencil, and hence will bring out the structure of a fine grating more readily than a direct pencil of light.

The sum $\alpha + \alpha_1$ acquires its smallest value when $\alpha = \alpha_1$. In this case we find from (6) that

$$n_1 \sin \alpha_1 = \frac{\lambda}{2 e_1} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

Supposing the angle α_1 in (7) to be equal to that in (1) and that it conforms to the greatest available aperture of an objective then

$$e_1 = \frac{e}{2} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

The application of oblique illumination enables us accordingly to resolve a grating which is twice as fine as compared with a grating seen under direct illumination.

We may now proceed to investigate what happens when a fine grating is being viewed with the iris-diaphragm fully open below the condenser. We shall now have both oblique and direct illumination. The latter, from what has been said, does not contribute to the resolution of the grating. Hence with the iris-diaphragm opened to its full extent the image will merely appear transfused with a general brightness, which has the effect of drowning in light the image of the structure of the grating. Clearly then, an extremely fine grating should not be viewed with the iris-diaphragm opened to its full extent, and the latter should be replaced by a stop having two small apertures arranged on a diameter at right angles to the bars of the grating.

So far we have supposed the object to be in the form of a grating, and in the case of an excessively fine grating we have increased the resolving power of a microscope by displacing the opening of the iris-diaphragm below the condenser at right angles to the ruling of the grating. We will now suppose that the object consists of very fine squares, in which case we are dealing with two gratings crossing each other at right angles. If, accordingly, we were to displace the pinhole stop at right angles to one system of bars, we shall only see this system clearly, whilst the other will merely give rise to a general illumination. On the other hand, if we displace the pinhole stop at right angles to the other system of bars the condition will be reversed. To view the structure of an object consisting of a system of extremely fine squares it will therefore be necessary to place under the condenser a stop having four apertures situated at the extremities of two diameters at right angles to the two component gratings.

By an easy step we may now pass on to a still more complex object. In this way we should have to make use of an ever increasing number of pinhole stops, until we reach a state of things as it presents itself in dark-ground illumination, which supplies oblique illumination in all azimuths. We cannot, however, attempt to enter into this matter here.

What has been said may suffice to furnish a general idea of the principles of microscopic vision, to which we will merely append one or two supplementary notes.

An object, when viewed under the microscope will transmit direct as well as diffracted light. Of this total quantity of transmitted light, direct and diffracted, a certain portion will be able to enter the objective, the amount of which depends upon the aperture of the objective. Suppose we have two objects which within the limits of a given angle furnish precisely the same diffraction pencils and diffraction spectra, but with this difference that one of the objects sends forth diffracted light of the first order beyond that angle, whereas in the case of the other object there is no diffracted light of the first order beyond that angle. If either object be viewed under an objective having an aperture which conforms strictly to this angle the objective will fail to dislose any difference in the two objects as seen through the microscope. As soon, however, as we bring an objective having a higher aperture to bear upon the finer structure it will present a markedly different appearance by reason of the increased resolving power of the objective. Nothing of the kind will happen in the case of the other object, since the objective of lower aperture was able, as we have seen, to admit the whole of the diffracted pencils proceeding from the object.

It will thus be seen that under certain circumstances two entirely different objects when viewed under a given objective may present an identical appearance. This will always happen when the aggregate pencil of light proceeding from an object is cut down by the aperture of an objective in such a manner that it becomes no wider than the aggregate pencil of direct and diffracted light proceeding from the other object

This brings us to an extremely important conclusion.*

Whenever the aggregate pencil of rays of diffracted light proceeding from an object is cut down, either by the natural aperture of the objective or by artificially arranged stops, the microscope will always furnish an exact magnified image of a supposed object whose entire cone of diffracted pencils is quantitatively similar to that part of the actual diffraction spectrum which is admitted to the objective.

* Cf. Dippel, Handbuch der allgemeinen Mikroskopie, Vol. I, p. 139, Brunswick, Vieweg & Sohn.

Initial Magnifications of Objectives and Eyepieces, and Magnification of the Compound Microscope.

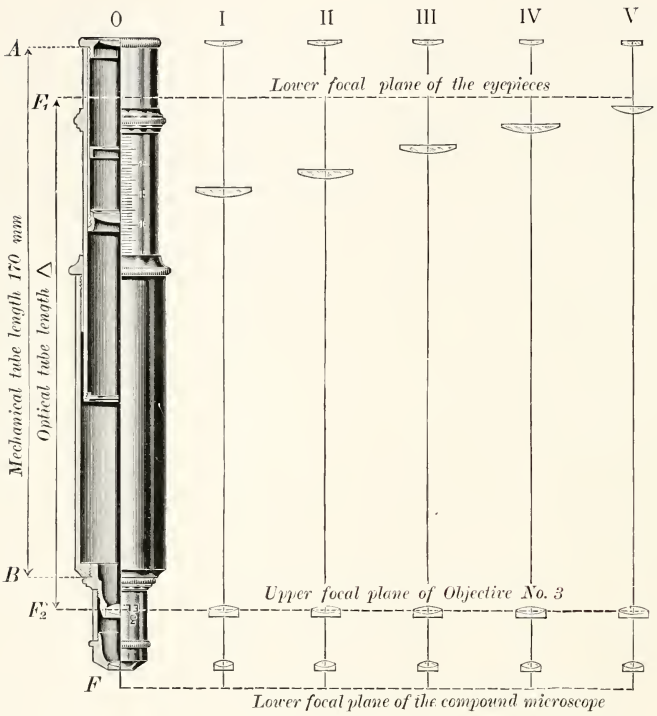

Fig. 9.

It may be useful to recall the following theorems in optics:

1. In any optical instrument having the object situated in its focal plane the corresponding image of the object is situated at infinity, i. e. the rays composing the pencils which proceed from the object to its image are parallel to each other when emerging from the optical system.

2. The linear magnification of the resulting image as produced by an optical instrument is numerically equal to its distance from the nearer focus of the instrument divided by its focal length.

Now the image as seen in the compound microscope may be regarded as the result of two successive projections, one being due to the objective, the other to the eyepiece. The microscopic image as viewed by the eye is situated at infinity, hence the component rays of each pencil emerging from the eyepiece are parallel. They appear accordingly to proceed from an object at the focal plane F_1 of the eyepiece. This apparent object is nothing more or less than the image of the actual object formed by the objective in that plane.

As before, we will denote the distance $F_1\ F_2^*$ (Fig. 9) of this image from the posterior focal plane of the objective with Δ. The initial magnification of the objective may then be expressed by the formula

$$M_{ob} = \frac{\Delta}{F_{ob}},$$

F_{ob} being the focal length of the objective.

With objectives differing in focal length and in their optical formula the position of the posterior focal plane F_2^* of the objective varies considerably, which causes the optical tube-length to assume widely differing values. With objectives of low power F_2^* lies a considerable distance above the objective towards the eyepiece, so that in their case the optical tube-length Δ is generally small, whereas high power objectives have their focal planes F_2^* generally close to the back lens of the objective, frequently even within the objective itself. This being so, the magnifications of objectives of different types may vary widely though there may be no difference in their focal length and in the mechanical tube-length $A\,B$.

The subjoined Table supplies for several Leitz objectives the calculated values of their focal lengths F_{ob}, the optical tube-length Δ and the resulting initial magnification

$$M_{ob} = \frac{\Delta}{F_{ob}}$$

Leitz Objective	Focal Length F_{ob}	Optical Tube Length $\triangle$	Initial Magnification $M_{ob}=\frac{\triangle}{F_{ob}}$	Formula of Objective	
1	40 mm	126 mm	3.2	Two positive doublets	
1a	24 „	75 „	3.1	Negative front and positive back doublet	Distance between doublets 14—30 mm
2	24 „	141 „	5.9	Two positive doublets	
3	16.2 „	167 „	10.3	Two positive doublets	
6	3.95 „	182 „	46.0	Hemispherical front lens and two positive doublets	All lenses in close proximity
1/12"	1.85 „	187 „	101.0	Oil-immersion. Hemispherical front lens with meniscus and two positive doublets	

From the Table it will be seen that the optical tube-length of the objectives specified therein varies within the limits of 75 and 187 mm.

It should be noted that Objective 1 a, by reason of its peculiar formula, has the same magnification as Objective 1, despite the fact that its focal length is much shorter; on the other hand, Objective 1a has the same focal length as Objective 2, yet the initial magnification of the latter is nearly twice as high as that of the former.

The initial magnification of the eyepieces is determined in a similar manner to that of the compound microscope (p. 10). In this case the image formed by the objective plays the same part as did the object in the compound microscope. Since the image is situated in the focal plane of the eyepiece the emerging pencils consist of parallel rays, which are readily brought to a focus on the retina of the eye.

Thei eyepiece magnifications may accordingly be expressed by the relation

$$M_{oc} = \frac{250}{F_{oc}}$$

The total magnification M of the compound microscope is the product of the two component magnifications, i. e.

$$M = M_{ob} \times M_{oc} = \frac{\Delta}{F_{ob}} \times \frac{250}{F_{oc}}$$

Since on page 10 it was shown that the total magnification s f $= \frac{F_{ob} \times F_{oc}}{\Delta}$, we may write $M = \frac{250}{f}$ which agrees with the expression on that page.

The subjoined Table gives the resultant magnifications as computed for a few Leitz objectives and eyepieces:

Eyepieces		Objective 1a	3	6	1/12"
No.	M_{oc}	$M_{ob} = 3.1$	10.3	46.0	101.1
0	4	12.5	41	184	403
I	5	15.6	51	230	503
II	6	18.8	62	277	605
III	8	25.0	82	368	805
IV	10	31.3	103	461	1009
V	12	37.6	123	553	1210

These computed values agree, of course, with those obtained by direct measurement, viz. by dividing the actual length of a scale into the length of its magnified image measured at a distance of 250 mm (cf. p. 11). When carrying out the measurement care should be taken to maintain the correct optical tube-length, to secure which the mechanical tube-length should always be $AB = 170$ mm. This length should be measured from the shoulder of the objective screw to the upper edge of the tube, and may readily be established with the aid of the scale engraved upon the draw-tube.

The Illumination.

Formerly the illumination was furnished simply by a plane and concave mirror. It constitutes still the entire illuminating apparatus of the smallest stands, and even on the larger instruments

nothing more elaborate is used in conjunction with low power objectives. The plane mirror should be used with low magnifications, say up to 100 diameters, whereas higher magnifications demand the use of the concave mirror. The plane mirror supplies parallel light, the concave mirror convergent rays included within an angular aperture of 40^0 or having a numerical aperture of about 0.34.

Higher magnifications call for the use of a condenser, of which there are three viz.

the two-lens condenser (Fig. 10),
the three-lens condenser (Fig. 11),
and the aplanatic condenser (Fig. 12).

Light is directed into these condensers by means of the plane mirror, as a rule; only when the source of light is very near to the mirror preference should be given to the concave mirror.

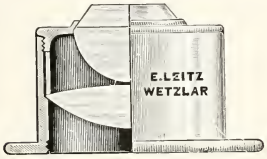

Fig. 10.
Two-lens Condenser
N. A. 1.20.

Fig. 11.
Three-lens Condenser
N. A. 1.40.

Fig. 12.
Aplanatic condenser
N. A. 1.40.

The two-lens condenser suffices in the great majority of cases. It has an aperture of 1.20, and when employed as an immersion condenser furnishes light comprising an angle of 104^0.

The three-lens condenser has a numerical aperture of 1.40 and furnishes an angular aperture of 134^0. It may be used with advantage as a means of bringing extremely wide or very oblique pencils of light to bear upon an object.

The same applies to the aplanatic condenser, whose numerical aperture is likewise 1.40. Being, however, corrected for both spherical and chromatic aberration, it furnishes a uniform and colourless illumination, which is a matter of importance especially in photo-micrography.

The illuminated area of the object may be circumscribed and all adventitious light cut off by means of a wheel diaphragm, interchangeable cylindrical stops,

or an iris-diaphragm. Large stops are suitable for low powers whilst small stops will generally be required for high powers. The iris-diaphragm, when used in conjunction with dry lenses should not be more than half open, whilst the full aperture should be employed with oil-immersion objectives and during the observation of finely granular objects only.

In addition to the ordinary direct illumination means are provided for the application of the principle of oblique illumination. To obtain oblique illumination with a microscope stand fitted with cylinder diaphragms the sleeve with its stops should be removed or, if the stand has a wheel-diaphragm, this should be turned so as to place its widest opening in the centre, and the mirror should be placed in an extra-axial position and so directed that the light reflected from it may fall obliquely upon the object. With an iris-diaphragm this is very simply done by placing the iris-diaphragm in an eccentric position by means of the pinion head at the side of the diaphragm carrier. By rotating the eccentrically placed diaphragm light may be thrown upon the object from any desired azimuth.

By means of the rack and pinion motion the entire illuminating apparatus may be raised and lowered, whereby the condenser may be brought into the best position relatively to the plane of the object.

To work without condenser it is only necessary to swing out the diaphragm carrier together with the iris-diaphragm and to withdraw the condenser from the sprung sleeve below the stage, replacing it by a cylinder-diaphragm. In the case of stands fitted with a stage iris-diaghragm and swing-out condenser the iris-diaphragm carrier with the lower iris-diaphragm should be swung aside, the condenser should then be released by a slight pressure on a projecting button and swung aside. Before returning the condenser to its original position care should be taken to ensure that the stage diaphragm is opened to its full extent, which may be done with the aid of the lever fitted to its mount. The lower iris-diaphragm is adjustable by means of a button and regulates the admission of light to the condenser.

Diffused light as emitted by uniformly white clouds is better adapted for microscopical observation than the light derived from a blue cloudless sky. Direct sunlight should be avoided in general.

The illuminators of the *a* and *b* types have below the iris-diaphragm a recessed collar which receives discs of ground or coloured glass for use with artificial light. The discs are supplied with the illuminators. The illuminating apparatus *c* of intermediate size has a ring collar pivoted below the iris-diaphragm for the support of the discs. When the light is derived from a lamp a very pleasant illumination may be obtained by the use of a blue glass, or the light may be diffused by the interposition of a ground glass disc.

Objectives.

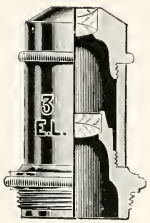

Fig. 13

Fig. 14

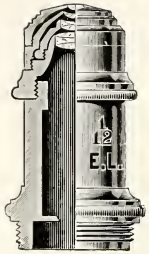

Fig. 15.

The above illustrations represent the three principal types of achromatic objectives.

The first figure shows an objective No. 3 of $^2/_3$ inch focus which is representative of the low and medium power *dry* lenses. Lenses of this type consist of two separate members, each of which is made up of two or three lenses cemented in contact, each member performing part of the optical correction.

The second figure is typical of the high power *dry* objectives which is made up of a hemispherical front lens and two members consisting of two or, in some cases, of three lenses in contact. The hemispherical front lens is the magnifying element proper, whilst the spherical and chromatic corrections are allotted to the two other elements.

In the third type, that of the $^1/_{12}$" oil-immersion lens, the hemispherical front lens is succeeded by a meniscus for the second member, whilst the third and fourth members are again composed of two or three cemented lenses and likewise perform the office of spherical and chromatic correction.

In practice it is essential to remember that the higher power objectives, from No. 5 upwards, are adjusted for cover-glasses 0.16 to 0.18 mm thick and for a tube-length of 170 mm. This tube-length, marked $T\,T_1$ on the diagram shown on p. 8, should be rigorously adhered to whenever high powers are being used. When a nosepiece is interposed between the tube end and the objective this should be allowed for and the draw-tube drawn out to 152 mm, whilst in the absence of a nosepiece the draw-tube should stand at 170. A discrepancy of 10 mm in the case of an oil-immersion lens mars its performance to an extent which virtually reduces it to that of a lens of indifferent quality.

There are three groups of objectives, respectively described as Apochromatic, Fluorite, and Achromatic Objectives, which differ mainly in their degree of colour correction.

1. Apochromatic Objectives. Apochromatic lenses surpass all others in the matter of colour correction, and involve as an essential factor in their construction the use of fluorite.

This mineral exhibits three optical properties of extraordinary value, viz.:

1. a high degree of transparency,
2. a low refractive index,
3. certain favourable features in its dispersion.

These combined properties are not to be found in any artificial optical medium, and it is not overstating the case to say that its introduction in 1886 marked a new era in the development of the microscope.

Its refractive index is 1.4339, and the reciprocal of the relative dispersion $\nu = \frac{n_D}{n_F - n_C} = 97$, whereas this value does not exceed 66.5 with any of the available optical glasses. Thanks to its low refractive properties it may be made to take the place of crown glass. The combination of fluorite with a glass of much lower refractive index than occurs in the usual types of flint glasses affords much more favourable conditions for the elimination of spherical aberration than the older combination of crown and flint glasses.

It so happens that the configuration of the spectrum as produced by a fluorite prism is very nearly similar in the relative position of its colour bands to that obtained with some of the glasses which are available for use in the place of the older flints. This property furnishes a means of rendering at least three optically significant colour rays homocentric, whereas with the older crown and flint pairs it was not possible to achieve a similar result with respect to more than two differently coloured rays.

Objectives which are achromatized with respect to two colours only exhibit residual dispersion or what is known as the *secondary spectrum*. This imperfection can be eliminated by bringing about the fusion of more than two colours with the aid of fluorite.

The high price of objectives of this latter class is due to their complex formula and to the practical difficulties involved in their production, and in a large measure also to the scarcity of fluorite of a quality adapted for optical purposes.

With many objects the absence of the secondary spectrum from the image produced by the apochromatic objectives endows it with a degree of brilliancy which cannot be attained with any of the

achromatic objectives. Fine butterfly's scales and diatoms are objects which never fail to demonstrate the beautiful properties of apochromatic lenses. The highest achievement in the construction of microscopic objectives is embodied in the 2 mm apochromatic oil-immersion lens having a numerical aperture of 1.40.

Though the apochromatic lenses bring rays of different colours to a focus in an ideal manner it is not possible to entirely remove the residual difference in the position of the focal planes for different colours, in consequence of which the resultant images are not strictly coincident. This overlapping of the images gives rise to colour fringes at the edge of the field. To eliminate this defect a special series of eyepieces has been introduced which are intentionally endowed with the opposite chromatic defect, or overcorrected, so that they may exercise a compensatory influence, hence the name *"Compensating Eyepieces"*. The formula of these eyepieces admits of higher magnifications than is practicable in the case of Huyghenian Eyepieces, which are however available for use with the apochromatic objectives. The advantages resulting from the elimination of marginal colours through the instrumentality of the compensating eyepieces is not so strikingly apparent as might be expected, nor will this be so until it has become possible to construct an entire range of objectives giving an absolutely flat field with a perfectly uniform degree of sharpness at all points from the centre to the edge (see page 33).

2. Fluorite objectives. This class embraces objectives which involve a higher order of correction and in which the secondary spectrum is greatly diminished without too widely departing from the simple formula of the achromatic objectives. This higher degree of correction has been realized by taking the fullest advantage of the extensive choice of optical glasses produced at the Glass Works of Messrs. Schott & Gen., of Jena, and by the judicious use of fluorite which enters into their construction.

In the endeavour to ensure a markedly higher degree of colour correction than that obtaining in the achromatic objectives it has, however, not been possible, as the name given to these lenses implies, to entirely dispense with the use of fluorite. This class comprises the dry lenses 6a, 7a, 8, and 9, and the oil-immersion lenses $^1/_{12}$"a and $^1/_{16}$". In their optical performance the fluorite lenses come very near to the objectives of the apochromatic series. This applies in particular to Objectives $^1/_{12}$"a and $^1/_{16}$". The difference between these and the apochromatic lenses of similar focus is so slight that only an expert viewing a specially selected test object will be able to appreciate the chromatic superiority of the 2 mm apochromatic objective. Objects viewed under dark-ground

illumination show the superiority of the apochromatic and fluorite lenses in a peculiarly striking manner, since this mode of illumination accentuates the chromatic imperfections of the achromatic lenses. Apart from the high power apochromatic lenses the fluorite objectives are therefore recommended for observation under dark-ground illumination.

3. Achromatic objectives. For all practical purposes and general scientific investigations the achromatic lenses are all that can be desired, and there is as yet no likelihood that they will be generally superseded by the more perfect type of lenses. This appears all the more improbable when it is borne in mind that during the last few years considerable progress has been made in the production of optical material, in consequence of which the achromatic lenses have been greatly improved in the matter of spherical and chromatic correction as well as in the flattening of the field; furthermore, the difficulty, not to say impossibility, of obtaining a sufficient supply of optically useful fluorite will remain a formidable obstacle in the way of any extensive manufacture of objectives involving its use. The improvements which have been made in achromatic lenses as regards the flatness of the field have greatly extended their utility in photo-micrography.

Eyepieces.

1. The Huyghenian Eyepieces are available for use with objectives of every type, though they are primarily designed for use with the achromatic and fluorite lenses.

The entire series comprises now 6 rationally graded eyepieces, respectively numbered 0, I, II, III, IV, V and giving eyepiece magnifications which for the distance of normal vision, i. e. 250 mm or 10 inches, magnify 4, 5, 6, 8, 10, 12 diameters, from which it follows that the magnifications of Eyepieces 0, I, II and III, IV, V are in the arithmetical progression 4 : 5 : 6. It will also be seen that the higher eyepieces III, IV, V produce double the magnification as compared with the lower eyepieces 0, I, II, whilst the highest eyepiece V has 3 times the magnifying power of the lowest eyepiece 0.

They are so computed as to cause the lower principal focus F_1 in the diagram Fig. 9 on page 24 for all eyepieces to be in the same plane, so that an adjustment which has been made with any one eyepiece will also hold good for the others. An interchange of eyepieces does not therefore necessitate re-focussing.

Here we must draw attention to a feature with which every microscopist is familiar but which he may not be able to interpret correctly. We allude to the fact that with high

power objectives it is not possible to focus sharply the entire surface of the field of view at the same time. It is found that when the centre is sharp the edge of the field is not clearly defined, and vice versa. It is, however, an error to conclude that this arises from a defect in the objective, on the contrary it is an inherent property of all lenses of higher powers and is known as the curvature of the image, as we shall briefly explain.

An object point situated on the axis has its corresponding image point likewise situated on the axis. The plane passing through this image point is called the *plane of the image*. If the image were flat in reality, then the images of all points situated outside the axis would be situated in this same plane. In general this is not the case; in fact, the image points do not lie in the plane of the image but on a surface which is more or less curved and which is tangential to that plane. All efforts to remove this evil have so far proved of no avail.

2. Compensating Eyepieces. These have been described on p. 32.

Focussing.

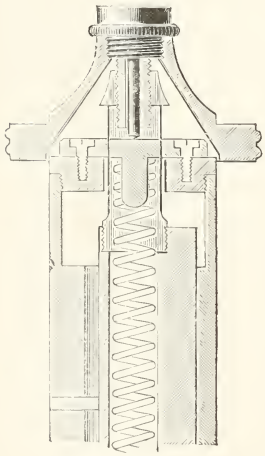

Fig. 16.

In all Leitz stands of large and intermediate size the mechanism for the coarse adjustment consists of a rack and pinion gear, and the smallest stands only are provided with a sliding tube for this purpose. In the latter case the adjustment should be made by imparting to the tube a gentle up and down screwing motion.

The fine adjustment is obtained by means of one or the other of two essentially different devices. One of these is of the nature of a vertical micrometer screw contained within the body pillar, whilst in the most recent patterns of the types A, B, C, D, E, F, G and H the slow motion is derived from a horizontal tangent screw operated by externally projecting milled heads. In the former type of slow motion mechanism, as shown in Fig. 16, the movable part slides upon a prismatic upright with a minimum of friction, yet without the least vibration. The prism is bored and contains a spiral spring which forces the movable part together with the barrel of the microscope vertically upwards, whilst a slot in the cylindrical head of

the prism serves to limit the amount of the vertical motion. The downward movement against the tension of the spring is produced by the micrometer screw, which, instead of acting directly upon the movable part contains in its axial bore a cylindrical steel pin, the object being to transmit a purely axial motion without setting up any tendency of a rotary kind, and thereby to render the motion as delicate and smooth as possible. The range of motion of the micrometer screw is about 5 mm, the pitch of the screw is $^1/_2$ mm and each of the 50 divisions of the head reads accordingly 0.01 mm. Should it become necessary to oil the micrometer screw it should be screwed out entirely, cleaned with benzol, and re-oiled, preferably with the finest bone-oil.

In the other type of the fine adjustment mechanism, as shown in Fig. 17, the milled heads by which it is operated are situated below the large milled heads for the rack and pinion gear. The central enclosed part of the spindle which carries the milled heads has cut upon it a worm screw, which engages into a worm wheel. A spiral spring exercises a continuous pressure upon one of the bearings of the worm spindle and thereby eliminates lateral play. The worm spindle carries a heart-shaped cam upon which runs a steel roller. The latter is made to bear upon the cam by the weight of the tube and the additional slight pressure of a weak spiral

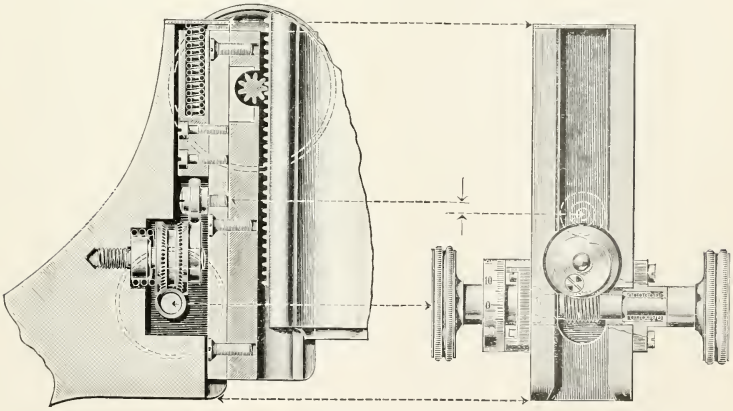

Fig. 17.

spring. At the periphery the cam is bounded by two symmetrical spiral surfaces, so that equal angular displacements of the cam produce equal linear displacements of the crest of the cam. This vertical motion is transmitted through the roller to the tube carrier. The total vertical range of motion of the spiral cam is 3 mm, and since the worm wheel has 60 teeth, it follows that half a revolution of the wheel, i. e. a rotation through 30 teeth, will

cause the tube to rise or fall through a distance of 3 mm, and hence a rotation through one tooth will cause the displacement to be $^3/_{30} = 0.1$ mm. A displacement of the wheel from one tooth to the next requires a complete rotation of the drum with its 100 divisions, so that the displacement of the drum through one division gives to the tube a vertical motion of 0.001 mm or 1 $\mu = {}^1/_{2500}$ of an inch.

Apart from the slowness and the sensitiveness of its motion this mechanism possesses other practical advantages. From the description it will be seen that the motion is continuous, in that the tube rises and falls alternately through a range of 3 mm whilst the milled heads are being turned an indefinite number of times. Should it so happen that the motion reverses just when the object is on the point of coming into focus it will, of course, become necessary to provide a fresh range of motion by raising the tube with the aid of the rack and pinion, placing the cam in its median position, and roughly focussing with the rack and pinion in the usual way, that is to say, the object should be brought into view and roughly focussed by means of the rack and pinion and then sharply focussed with the fine adjustment. It is immaterial whether the position obtained by rough-focussing is above or below the plane of the critical focus, as half a turn of the fine adjustment milled head, or less, suffices to show whether the image is vanishing or becoming more clearly defined. Since 30 turns are required to reverse the motion the cam is not likely to reach its upper or lower limits excepting on rare occasions.

The risk of crushing an object by an accidental impact of the front lens with the object is all but nil; for in the event of the objective coming in contact with the cover-glass it will, together with the tube, merely rest upon the latter, which will readily bear the slight pressure due to the weight of the tube and the tension of the buffer spring above the cam roller. At the side of the tube and at two points of the fixed part of the limb the highest and lowest points of the micrometer motion are marked by two terminal lines and an index.

The subjoined Table gives the working distances for the entire series of objectives, i. e. the distance between the front lens and the object, which may prove helpful to the beginner and with which he will do well to familiarize himself.

With the lower powers the distances given in the Table will enable him to place the lens very nearly in its correct position, whilst high power objectives should be made to all but touch the cover-glass, which may be accomplished by glancing between the object and the front lens. A little care will be needed, as the objective or cover-glass may be damaged if the tube be racked down too rapidly or too forcibly. When the objective has been thus brought close to the object the tube should be slowly racked back until the image comes into view.

	Objective	Focal Length	Numerical Aperture	Free Working Distance	Diameter of field of view with Eyepiece O.	
Achromatic Objectives	1*	42 mm	0.08	40 mm	8.5 mm	Dry Series
	1	40 „	0.11	34.5 „	7 „	
	1a	33—24 „	0.05—0.07	14.0—2.0 „	6.5—10 „	
	2	24 „	0.21	16.0 „	4 „	
	3	16.2 „	0.30	5.5 „	2.1 „	
	3a	13.0 „	0.40	3.2 „	1.6 „	
	4	10.0 „	0.47	2.0 „	1.1 „	
	5	5.4 „	0.77	0.76 „	0.70 „	
	6	4.0 „	0.82	0.42 „	0.48 „	
	7	3.2 „	0.85	0.29 „	0.35 „	
	10	2.1 „	1.20	0.11 „	0.23 „	Water Immersion
	1/10	2.8 „	1.30	0.28 „	0.34 „	Oil Immersion
	1/12	1.8 „	1.30	0.17 „	0.24 „	
Fluorite Objectives	6a	4.0 „	0.82	0.38 „	0.48 „	Dry Series
	7a	3.2 „	0.85	0.27 „	0.35 „	
	8	2.6 „	0.87	0.22 „	0.30 „	
	9	2.2 „	0.87	0.16 „	0.24 „	
	1/12a	1.8 „	1.32	0.12 „	0.24 „	Oil Immersion
	1/16	1.6 „	1.32	0.09 „	0.20 „	
Apochromatic Objectives	16	16.0 „	0.30	4.3 „	0.94* „	Dry Series
	8	8.0 „	0.65	0.96 „	0.46* „	
	4	4.0 „	0.95	0.21 „	0.23* „	
	3	3.0 „	0.95	0.15 „	0.16* „	
	2	2.0 „	1.32	0.13 „	0.12* „	Oil Immersion
	2	2.0 „	1.40	0.07 „	0.12* „	

When Eyepiece III takes the place of Eyepiece O the diameter of the field of view becomes diminished to **78.5** % as compared with the area of the field of view of the latter.

* Measured in conjunction with compensating Eyepiece 8.

Revolving nosepiece and objectives supplied together are adjusted in such a manner that the focussing of one lens places the other lens or lenses when rotated into position at approximately the correct distance, a slight turn of the micrometer screw being all that is necessary to complete the adjustment, provided, of course, that the tube-length is properly adjusted to 170 mm.

Micrometric Measurements under the Microscope.

The linear dimensions of a microscopic object in the plane of the stage may be measured with the aid of an eyepiece micrometer, when calibrated by a stage micrometer either of which is of the nature of a fine scale ruled upon a glass plate. The object micrometer lies in the plane of the object in the place of the latter, whilst the micrometer eyepiece lies in the plane of the eyepiece diaphragm. By focussing a stage micrometer with a given combination of an objective and eyepiece at a definitely fixed tube-length and comparing the two micrometer scales it is easy to find the actual length in the plane of the object which corresponds to one scale division of the eyepiece micrometer. This quantity is called the micrometer value of the scale, and it will be readily understood that it holds good so long only as an invariable tube-length is adhered to. The Table overleaf gives the micrometer values of the eyepiece micrometer for achromatic and fluorite objectives in combination with Eyepiece II and for apochromatic objectives with Compensating Eyepiece 4. These values hold good for a tube-length of 170 mm, which must be rigorously adhered to, and for an eyepiece micrometer scale divided into $^1/_{10}$ ths of a millimetre. If the micrometer scale is made up of $^1/_{20}$ ths of a millimetre the tabulated values should be halved.

Example. Let a scale of *Hipparchia Janira* as seen with Objective No. 6 cover 50 divisions of the scale longitudinally and 18 divisions transversely. Its actual length will then be $50 \times 0.0037 = 0.185$ mm and its breadth $18 \times 0.0037 = 0.067$ mm.

Supposing a valve of *Pleurosigma angulatum* measured with objectives 4, 6, and 7 to cover 29, 71, and 90 divisions respectively, then the measurements of its length as obtained with these objectives give the following absolute longitudinal dimensions:

with Objective 4: $29 \times 0.009 = 0.261$ mm,
„ „ 6: $71 \times 0.0037 = 0.262$ „
„ „ 7: $90 \times 0.0029 = 0.261$ „ .

Table of Micrometer Values.

1 μ = 0.001 mm

Achromatic Objectives		Fluorite Objectives	
Objectives	Micrometer Values measured with Eyepiece II	Objectives	Micrometer Values measured with Eyepiece II
1*	62 μ	6a	3.7 μ
1	54 μ	7a	2.0 μ
1a	80 — 54 μ	8	2.4 μ
2	30 μ	9	1.9 μ
3	17 μ	1/12a	1.7 μ
3a	13 μ	1/16	1.4 μ
4	9 μ	**Apochromatic Objectives**	
5	5.4 μ	Objectives	Micrometer Values measured with Compensating Eyepiece 4.
6	3.7 μ	16 mm	16 μ
7	2.9 μ	8 „	8 μ
10	1.8 μ	4 „	4 μ
1/10	2.6 μ	3 „	3 μ
1/12	1.7 μ	2 „	2 μ

It is usual to adopt the micron (μ) or 0.001 millimetre as the linear unit measurement.

When very accurate results are required it is advisable to use a screw micrometer eyepiece instead of an eyepiece scale micrometer. The former has a movable index within the plane of the diaphragm, i. e. within that plane which contains the real image produced by the objective. The pitch of the screw is such that one division of the drum corresponds to a linear displacement of the index amounting to 5 μ.

To render the apparatus available for measurements it requires, like the ordinary eyepiece micrometer, calibration by means of a stage micrometer, the operation consisting in determining how many divisions of the drum correspond to one division of the stage micrometer. The value so found is largely dominated by the tube-length, and hence it will be necessary to calibrate the drum readings for every new series of measurements.

Linear dimensions at right angles to the plane of the object or along the axis, in other words the depth of an object, may be measured with the aid of the micrometer divisions of the fine adjustment. As stated on page 36, each division of the graduated drum corresponds to a vertical displacement of 1 μ. The difference between the readings obtained for two points focussed in succession one above the other gives the distance between them, provided one is vertically above the other, and preferably close to the axis. This is an important proviso as the curvature of the image is likely to vitiate the measurement.

General Hints.

Before withdrawing the microscope from its cabinet or case note how the instrument is accommodated therein.

The instrument is best carried by its upper frame. It is so fitted in its case that this part may be taken hold of.

When a microscope has been in a cold room it should not be used immediately after in a warm room, since vapour would condense upon the lens surfaces.

Novices should accustom themselves from the first to place the eye as close to the eye-lens as possible and to work with either eye alternately. The unemployed eye should not be closed but remain open in a passive state of accommodation.

All superfluous light should be excluded by the proper use of stops. Excessively bright light interferes with the observation and is injurious to the eyes.

Observation should always be commenced with low power objectives and followed up with higher powers as the nature of the

object may demand. It is also advisable to exercise moderation in the use of high eyepieces. These are better adapted for measuring and counting than for observation.

Eyepieces and objectives require occasionally thorough inspection to make sure that their surfaces are clean. The eyepiece has an eye-lens and a field-lens in separately detachable screw collars, which renders it easy to clean them.

The front and back surfaces of objectives may without risk be cleaned by the owner himself. Internal surfaces should be examined with a hand lens. Any cloudiness or opacity which may be noticed should be removed with the utmost care, and frequently it will be advisable to entrust the maker with this task. Formerly objectives, from whatever source, were liable to undergo devitrification, in consequence of lack of experience regarding the properties of the materials employed. Whenever this contingency arises objectives made at the Leitz Works will be exchanged free of charge for objectives in which such glasses only are used which have stood the test of time. It should, however, be added that in recent years these occurrences have all but vanished.

Dust is removed from the lens surfaces by means of a fine dry camel hair brush and by blowing lightly whilst brushing. If this fails to remove the dust the lens surfaces may be breathed upon and gently wiped with a clean soft piece of linen. Particles of dirt adhering very firmly should be removed with linen damped with alcohol. When examining objects treated with chemical reagents great care must be taken that they do not come in contact with the lens surface. Should this nevertheless happen the lens should at once be rinsed in water and carefully wiped. The use of rather large cover-glasses is the best means of preventing such occurences.

No futile attempts should be made to obtain other powers by unscrewing optical components, every objective being of the nature of an inconvertible system of interdependent units.

Before and after using the microscope it is wise to inspect the objectives and eyepieces and to clean them at once if necessary. The stand, too, requires occasional attention. Its rack and pinion, fine adjustment and other important parts should occasionally be carefully cleaned and sparingly lubricated with oil which should be perfectly free from acid, such as pure bone-oil.

The stand should be cleaned with soft linen or chamois leather and in so doing the leather should be passed along the grain of the polish and not across it. On no account should alcohol be allowed to come in contact with the yellow lacquer on the stand,

whilst benzol or xylol may be used for this purpose with perfect impunity.

Any discolouration on the vulcanite stage caused by benzol etc. may be removed by rubbing with oil.

Separate cloths should be used for objectives, eyepieces, and the stand, and they should be kept in a dust-proof receptacle.